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Bioactive Yields of Fermented Rice by Ten Generation of *Monascus purpureus* JMBa after Six Years Storages by Using HPLC and Spectrophotometer

Nandang Suharna, Nilam F. Wulandari, Titin Yulinery, Evi Triana, Novik Nurhidayat

Research Center for Biology, Indonesian Institute of Sciences, Cibinong Science Center,
Jl. Raya Jakarta-Bogor, Km.46, Cibinong 16911, Bogor-Indonesia

Abstract

The objective of this study was to know the stability of bioactive production of *Monascus* fermented rice (MFR) by *Monascus purpureus* JMBA after six years storages by using HPLC and Spectrophotometer. The results showed instability in the use of *M. purpureus* culture based on the bioactive yield analysed. Despite there were still bioactive which was detected by HPLC in FMR after six years storages, however, this results also indicated a tendency towards lowering red or yellow pigment and lovastatin yields of the fermented products over the tenth descendants. Further study is needed to clarify instability in producing bioactive by *M. purpureus* JMBa, MFR product in particular.

Keywords: bioactive, instability, *Monascus purpureus*, fermented rice, storages, HPLC

1. Introduction

M. purpureus are the major *Monascus* species known. *Monascus* strains are commonly found from traditional oriental food [1]. Traditional fermentation in China have involved the use *Monascus* as important fungi for thousands years ago. Special benefit of food and its application as drug substances have been documented in ancient history [2]. *Monascus* was included in family Monascaceae, Eurotiales. *M. pilosus*, *M. purpureus* and *M. ruber* by Hawksworth *et al*. [3] before re-classification based on phyllogeny those placed under family Monascaceae (Eurotiales, Eurotiomycetidae, Eurotiomycetes, Pezizomycotina, Ascomycota, Fungi [4].

Many recent researchs of *Monascus*'s secondary metabolites have been carried out mainly based on its potential in producing bioactive such as pigments, yellow, orange and red pigments [5, 6], antihypercholesterolemic agents, such as monacolin K and hypotensive agent, γ-amino butyric acid (GABA) [6, 7] and antibacterial substances including pigment and citrinin (as monascidin A) [8]. Monacolin K (known as Lovastatin, Mevinolin and Mevacor) is a secondary metabolite produced by *Monascus* and *Aspergillus* [9]. *Monacolin* K is inhibitor to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), an enzyme responsible for cholesterol biosynthesis [7]. Angkak or China red yeast rice or Monascus-fermented product has been known to reduce in significant the total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) concentrations in blood serum [10]. A report has been documented the use of angkak as traditional medicine and food and drink colorant in Asia and Chinese communities in North America [11].

This objective of this study was to know the stability of bioactive production of Monascus fermented rice (MFR) by *M. purpures* JMBA after six years storages by using HPLC and Spectrophotometer. It was important to know the stability of bioactive production by fungal culture used continuously and after six years storages. This study was mainly aimed at analysis of pigments (red and yellow pigment) and lovastatin production.

2. Materials and Methods

2.1. Angkak samples

Angkak samples were originated from angkak kept in jar bottle with volume 250 ml which were stored at 25°C for six years and hindered from sunlight. Each angkak samples (FMR F1, FMR F2, FMR F3, FMR F4, FMR F5, FMR F6, FMR F7, FMR F8, FMR F9, and FMR F10) was derived from ten generation of *M. purpureus* culture.

2.2. Spectrophometric analysis of pigments production

Analysis on red or yellow pigment was carried out spectrophotometrically by extraction of 0.05 g powdered angkak in 10 ml of methanol for 24 hours by using electric shaker, then filtered by using filter paper. The filtrate was measured for yellow pigment (λ=390nm) and red pigment (λ=500nm) by using Shimadzu Pharmaspec 1700 spectrophotometer.

2.3. HPLC analysis of lovastatin

Lovastatin was extracted from amount of 1 gr of powdered angkak with solution to 2 ml acetonitrile and 0,1 ml 0,1% phosphoric acid with 30 minutes incubation time. The solute was centrifuged at 10,000 rpm for 10 minutes at 4°C. To make concentrated, the supernatant was freeze dried and then diluted by mobile phase solution (acetonitrile + 0.1% phosphoric acid (65:35). HPLC analysis was completed by injecting 20µl and by using column C18 and detector UV λ235 nm with flow rate 1 ml/minute at 45°C.

3. Results and Discussion

All products still contained of red (Figure 2) and yellow pigment (Figure 3). The yield pigments varied amongst all products. But, overall products of the ten generations showed similar pattern between the red pigment and yellow pigment. It showed that product of F1, F3, F6 and F9 were the best product containing the both pigments. The rest were underrated. Moreover, product of F10 was the lowest. This result showed a tendency that over ten generation showed decreasing potency of the *Monascus* strain in bioactive production (Figure 2, Figure 3).

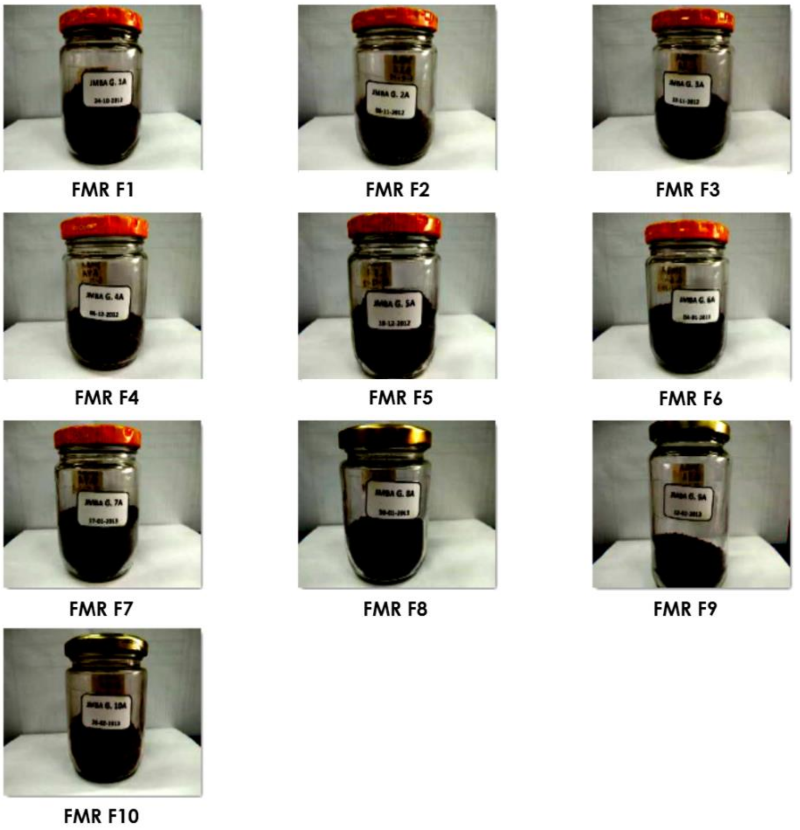

Figure 1. Sample of Angkak stored at 25°C for six years hindered from sunlight.

Srianta et al [12] reported that very high yield of the pigment and the color value at more 700 U/mL of fermentation broth can be reached in submerged fermentation. While in current industry scale, the color value of Monascus red pigment products can be reached at 10000 U/g ~ 15000 U/g.

Monascus pigments are polyketide components that those range in structure from tetraketides to octaketides. Typical classes contain the anthraquinones, naphthoquinones, hydroxyanthraquinones, and azaphilone structures, each of which reveals an array of color hues. [12, 13]. However, *Monascus* pigments mainly consists of three types of color components (yellow, orange and red) those are group of azaphilones mixture. Recently, more than 50 Monascus pigments have been found and characterized.

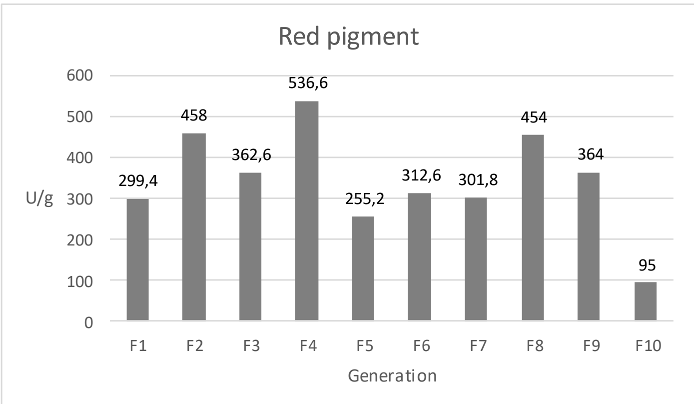

Figure 2. Red pigment yields of angkak of ten generations after 6 years storages.

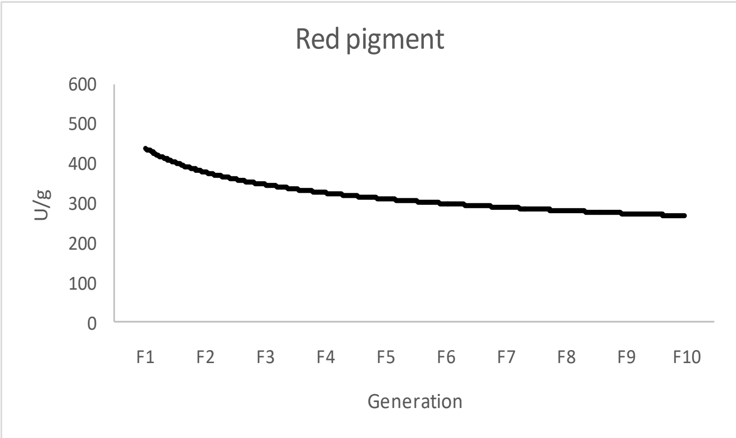

Figure 3. The Stability of red pigment yields of angkak over ten generations after 6 years storages.

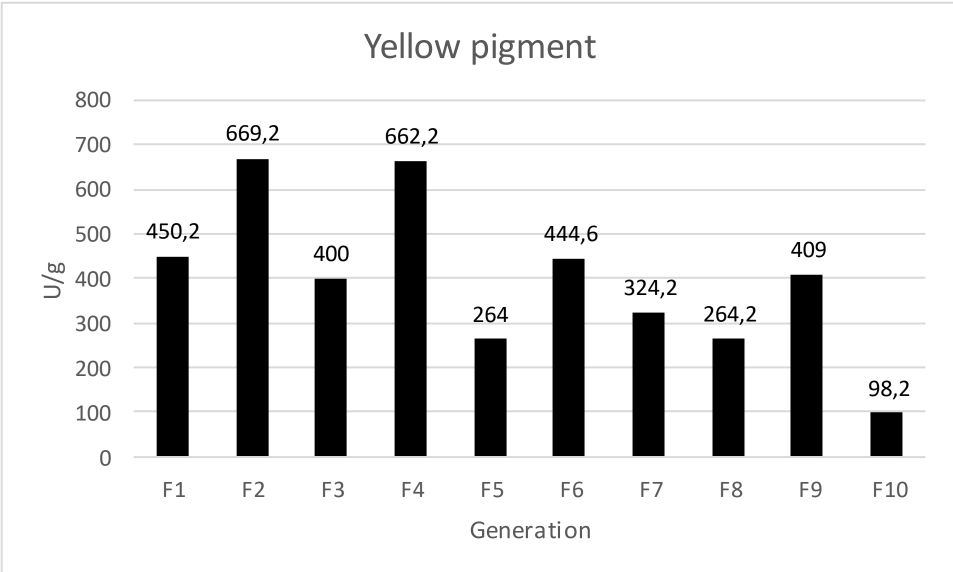

Figure 4. Yellow pigment yields of angkak over ten generation after six years storage.

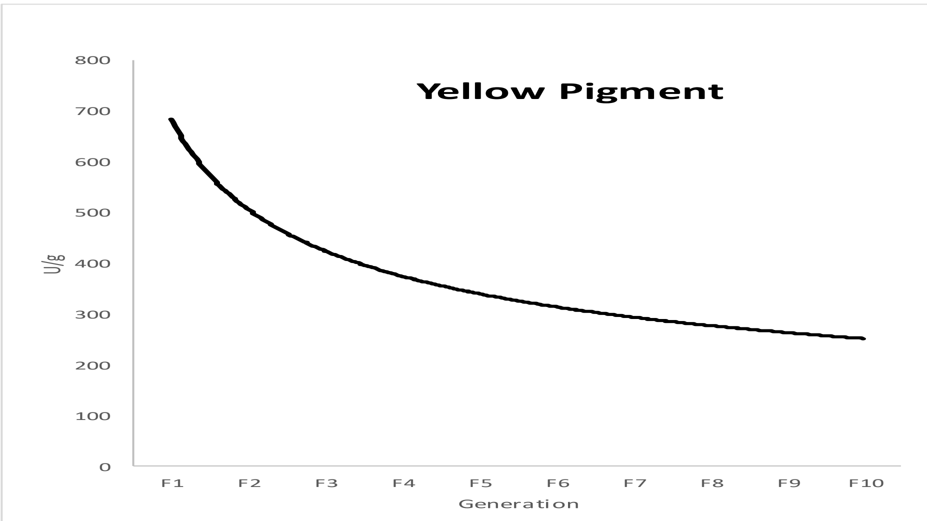

Figure 5. The Stability of yellow pigment yields of angkak over ten generations after six years storages.

Moreover, those of yellow *Monascus* pigments have been discovered their potential for anti-tumor, anti-diabetic, anti-oxidative stress, anti-inflammatory and anti-obesity bioactivities. The orange *Monascus* pigments also reported to show anti-cancer effects [14]. Many studies have shown that both *Monascus* pigments are derived from polyketide and the production and characteristics of pigments can be regulated by the medium compositions and culture conditions [14]. Furthermore, there was a report that fed-batch culture with proper nutrient source may deliver a better strategy for high mass cell growth. Studies using high concentration fermentation of *Monascus* pigments resulted the highest yield of biomass up to 28 g/L dry cell weight [15].

The result showed that lovastatin was detected from all products except for F8 and F10 (Figure 4). F3 showed the best and high lovastatin production. However, it was a tendency of reducing capacity of lovastatin production over ten generation.

It was also in interest that bioactive were still found in *M. purpureus* JMBa isolate after more than six years at room temperature (25°C) and hindered from direct sunlight.

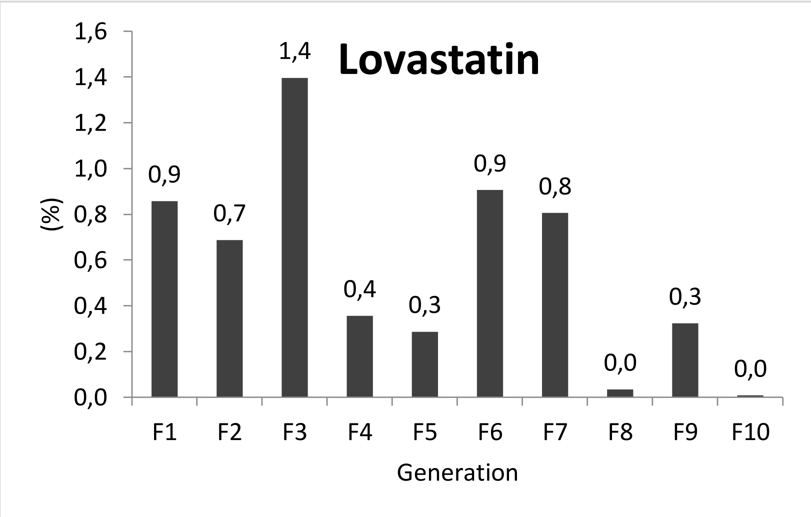

Figure 6. Lovastatin yields of angkak over ten generation after 6 years storage.

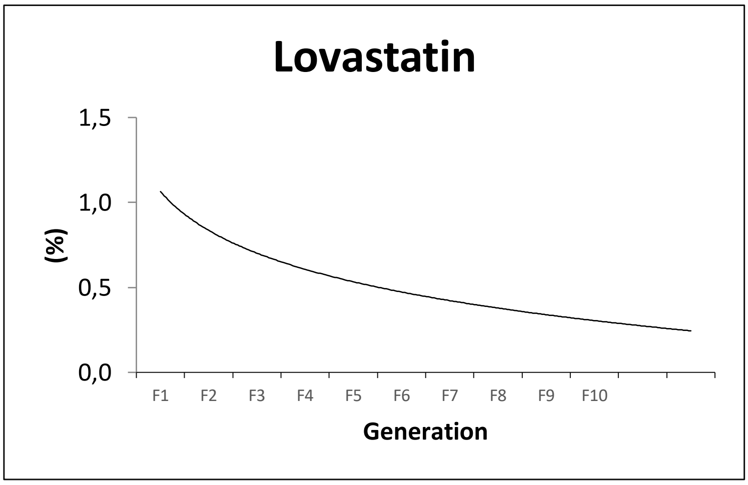

Figure 7. The Stability of lovastatin yields of angkak over ten generations after 6 years storages.

Table 1. Production of Lovastatin in solid state fermentations.

Fungal Strain	Lovastatin concentration	Substrate	Reference
M. purpureus Jmba	0.9-14 mg/g*)	Rice	This study
A. terreus ATCC 74135	0.26 mg/g	Rice straw	[16]
A. terreus JPM 3	0.98 mg/g	Wheat bran	[17]
A. terreus ATCC 20542	2.9 mg/g dry	Rice	[18]
A. flavipes BICC 5174	16.65 mg/ g	Wheat bran	[19]
M. purpureus MTCC 369	2.83 mg/g	Rice	[20]
A. terreus UV 1718	3.72 mg/g	Wheat bran	[21]

*) = from the 1st to 3rd generation after six years storage.

Table 1 shows that the *M. purpureus* Jmba strain could be considered as a good pigments and lovastatin producer among the other strains. However, the use of the fungal culture was good until the third generation (figure 5). The unstable production may be overcome by using a master culture which is preserved in freeze-dried form or kept in -80°C or in liquid nitrogen to secure the genetically stable strains [22]. The use of the preservation method applied is vital for industrial production providing genetic stable fungal strains [23].

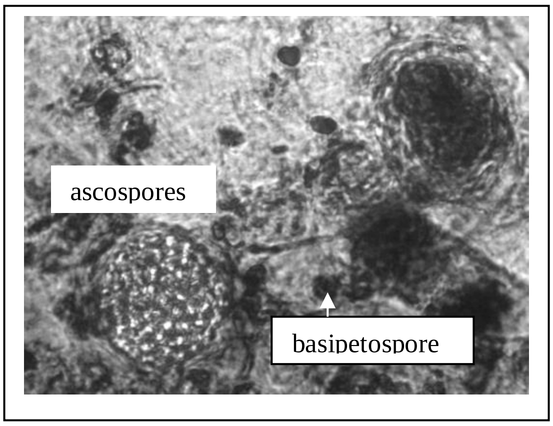

Figure 8. Compact myeclial mass of *Monascus purpureus* filling the rice at 1000x magnification.

Figure 8 is showing microscopic culture of *M. purpureus* Jmba which produce ascospores and basitopetospores. Mycelial masses are massively growing entire rice substrate. This indicates that the fungus grew very well and produces bioactives included pigments, lovastatin and others.

This result work showed that the storage over six years of angkak showed the bioactive content that seem did not change much. This study showed that the use *Monascus* starter continuously need much concern as the quality of the product was gradually reduced based on bioactives, such as pigment and lovastatin.

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4. Conclusion

The effective use of *M. purpureus* culture among ten generation for fermented rice production and its bioactive yield of the product after six years storage was analysed. There were obvious that there were a tendency on lowering red or yellow pigment and lovastatin yields of the fermented products among the use of the tenth descendants. However, high bioactive was still found in FMR after six years storages. Therefore, the storage condition may supported the stability of the bioactive studied such as pigments and lovastatin. Further study is needed to improve *M. purpureus* Jmba as the starter so better bioactive production stability will be achieved.

5. References

[1]. Sabater-Vilar, M, Roel F, Mass, RFM and Fink-Gremmels J.. Mutagenicity of Commercial Fermentation Products and the Role of Citrinin Contamination, *Mutat. Res.* 1999. 444: 7-16.

[2]. Özlem Erdoğrul. Review of The Studies on The Red Yeast Rice (Monascus purpureus). *Turkish Electron J. Biotechnol*. 2004;2:37–49.

[3]. Hawksworth DL, Pitt JI. A new taxonomy for Monascus species based on cultural and microscopical characters. *Aust. J. Bot*. 1983; 31(1):51–61

[4]. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon F, Eriksson OE, et al. A higher-level phylogenetic classification of the Fungi. *Glob. Biodivers*. 2007;111:509–47

[5]. Su Y-C, Wang J-J, Lin T-T, Pan T-M. Production of the secondary metabolites gamma aminobutyric acid and monacolin K by Monascus. *J. Ind. Microbiol. Biot.* 2003. 30 (1): 41–6. doi:10.1007/s10295-002-0001-5

[6]. Lin C C, T-C Li, and M-M Lai. 2005. Efficacy and safety of *Monascus purpureus* Went rice in subjects with hyperlipidemia. *Eur. J. Endocrinol.* 153: 679-686

[7]. Endo A, Komagata D and Shimada H. Monacolin M: A new inhibitor of cholesterol biosynthesis. *J. antibiot.* 1986. 39:1670-1673

[8]. Blanc P J Laussac, J P; Le Bars J, Le Bars P, Loret, M O, Pareilleux A, Prome D; Prome J C; Santerre A L; Goma G. Characterization of monascidin A from *Monascus* as citrinin. *Int. J. Food Microbiol.,* 1995. 27: 201–213

[9]. Endo A. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. *J. Antibiot.* 1979. 32: 852–854

[10]. Lee C-L, Hung Y-P, Hsu Y-W, Pan T-M. Monascin and ankaflavin have more antiatherosclerosis effect and less side effect involving increasing creatinine phosphokinase activity than monacolin K under the same dosages. *J. Agric. Food. Chem. 2013.* 61(1):143–50

[11]. Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff R M, Go VLW. Cholesterol lowering effects of a proprietary Chinese red-yeast. *Am J Clin Nutr*., 1999 69:231–6

[12]. Srianta I, Ristarini S; Nugerahani I; Sen SK; Zhang BB;Xu GR; Blanc PJ. Mini Review Recent research and development of Monascus fermentation products. *Int Food Res J.* 2014; 21(1):1–12

[13]. Liang B, Du XJ, Li P, Sun CC, Wang S. Investigation of citrinin and pigment biosynthesis mechanisms in monascus purpureus by Transcriptomic Analysis. *Front. Microbiol*. 2018; 9(JUN):1–11

[14]. Moussa LA, Azeiz AZA. Effect of media composition on citrinin and bio-pigments production by *Monascus ruber*. *J. Appl. Biol. Biotechnol.* [Internet]. 2017;5(02):104–9. Available from: http://jabonline.in/abstract.php?article_id=202

[15]. Chen G, Shi K, Song D, Quan L, Wu Z. The pigment characteristics and productivity shifting in high cell density culture of Monascus anka mycelia. BMC *Biotechnol*. [Internet]. 2015;1–9. Available from: http://dx.doi.org/10.1186/s12896-015-0183-3

[16]. Shu PY & Lin CH. Simple and sensitive determination of citrinin in monascus by GC-selected ion monitoring mass spectrometry. *Anal. Sci.* 2002. 18(3), 283–87

[17]. Jahromi MF, Liang JB, Ho YW, Mohamad R, Goh YM, Shokryazdan P Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation *J. Biomed. Biotechnol.* 2012:1–11

[18]. Jaivel N, Marimuthu P. Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus*. *Int. J. Eng. Sci. Technol.* 2010 2(7):2730–3

[19]. Wei P, Xu Z, Cen P. Lovastatin production by *Aspergillus terreus* in solid-state fermentation. *J. Zhejiang Univ. Sci. A.* 2007, 8:1521–6

[20]. Panda BP, Javed S, Ali M. Optimization of fermentation parameters for higher lovastatin production in red mold rice through co-culture of Monascus purpureus and *Monascus ruber*. *Food Bioprocess Technol*. 2010, 3(3):373

[21]. Pansuriya RC, Singhal RS. Response surface methodology for optimization of production of lovastatin by solid state fermentation. *Braz. J. Microbiol.* 2010 41:164–72

[22]. Alonso S. Novel Preservation Techniques for Microbial Cultures. In: Novel Food Fermentation Technologies. Food Engineering Series. Springer, Cham.: Ojha K., Tiwari B. (eds); 2016:7-33

[23]. Haynes WC, Wickerham LJ, Hesseltine CW. Maintenance of cultures of industrially important microorganisms. *Appl. Microbiol*. 1955. Nov;3(6):361